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Solanine Concentrations and Tuber Yields in the Netted Gem Potato as Affected by Certain Cultural Practices.

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## ABSTRACT

High concentrations of solanine in potatoes give them a bitter flavour and may even produce toxic symptoms if consumed. Though high solanine content is well-known to be associated with greening from exposure to light, investigations have shown that dangerously high solanine concentrations do occur in Netted Gem potatoes that are of excellent appearance and that show no visible greening.

To study the effect of certain field cultural practices on the occurrence of solanine in Netted Gem potatoes, two factorial experiments were designed in three replicates and continued for two seasons. The factors studied were two depths of planting with three levels of hilling, and two dates of planting with single and multiple eyes.

The cold ethanol extraction method used on the 1950 tuber samples was an arduous procedure giving unsatisfactory results, in which no treatment trends were evident. Dabbs' acidified ethanol Soxhlet extraction method applied to the quick-frozen 1951 samples not only reduced extraction time but gave satisfactory checks.

A high solanine variance due to replicates characterized both split-plot factorial experiments in 1951. In

one factorial it reached the 5 per cent level of significance, which indicated, in this experiment at least, that the variability of uncontrolled environmental factors in the field was greater than that due to the cultural treatments studied. In effect, though variance due to an interaction of planting dates with eye number was quite marked, the conclusion is that the cultural treatments studied made no significant difference in the occurrence of solanine in the tubers under the conditions of the experiment.

Concomitant tuber yield studies showed that:

(1) Normal planting gave consistently but not significantly greater yields than late planting in both years.

(2) No consistent differences existed in the yields obtained from single-eye and multiple-eye seed-pieces.

(3) Shallow planting resulted in yields equal to, or greater than, deep planting. Differences were not significant.

(4) There was no difference in yield between no hilling, low hilling or high hilling treatments.

THE UNIVERSITY OF ALBERTA

# SOLANINE CONCENTRATIONS AND TUBER YIELDS IN THE NETTED GEM POTATO AS AFFECTED BY CERTAIN CULTURAL PRACTICES

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

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# INTRODUCTION

Reports of bitter flavor in table potatoes are received by the Horticulture Division of the University of Alberta, Department of Plant Science, every year. These reports do not appear to be confined to any particular time of year nor to a particular area. The bitterness appears to be found mainly in the variety Netted Gem, which is the most widely grown variety in Alberta at the present time. This is a report of a study of the influence of certain cultural practices on solanine content in the Netted Gem variety.

# REVIEW OF LITERATURE

## History and Importance of the Variety Netted Gem

Before Luther Burbank left New England for California, where he became famous as a plant breeder, he had already selected a white-skinned potato which became known as Burbank's Seedling (25). The selection was made from an open-pollinated Early Rose potato, a pink-skinned variety. From the relatively smooth-skinned Burbank's

Seedling, a mutation or seedling arose which was deeply netted or darkly russeted. The latter became known as a distinct variety, and is now very widely grown under several names, including Russet Burbank, Idaho Russet, Idaho Baker, and Netted Gem (25, 48). It is now the leading variety for seed certification in British Columbia, Alberta and Saskatchewan (12). It is also the foremost potato variety in the four Northwestern States, as is shown in Table I, the figures for which were taken from the American Potato Yearbook 1951 (11).

Table I. Potato Production Devoted to the Variety Netted Gem

| | | | |
|---|---|---|---|
| Idaho | 95% | Washington | 65% |
| Oregon | 74% | Montana | 60% |

From the above facts it is clear that the economic importance of the Netted Gem variety is indisputable. What, then, are the qualities or factors contributing to its universal popularity in the West?

In Alberta, though far from being an ideal potato, the Netted Gem has several characteristics which make it a difficult variety to replace. It is scab-resistant, an important attribute in high-lime prairie soils. Its attractive shape and skin color, together with its shallow eyes and

short-season characteristic, combine to give it a good appearance when well-grown. The variety appears well-adapted to irrigation and keeps well in storage. In fact, Wright and Whiteman (56) found that Russet Burbank required an average of thirteen weeks to sprout even when stored at 50° F. In Idaho, it is highly esteemed as a baking potato (48), while in a test of the chipping quality of 33 Maine-grown varieties Netted Gem was one of the eight varieties that produced chips of outstanding attractiveness whether the tubers were stored at 40° F, then conditioned at 70° F, or whether stored at 55° F (57).

The above characters, together with its popularity in Idaho, and the resultant demand for Canadian Certified Seed, have firmly entrenched the Netted Gem variety in the Alberta potato-growing industry. Alberta growers of certified seed supply seedstocks to the commercial grower at home and abroad. How much the "popularity" of the Netted Gem variety in Alberta is attributable to consumer preference, and how much it has been thrust upon the consumer by the seed growers who cater primarily to the export trade in seedstocks and use the home table market as an outlet for surpluses, is a matter for conjecture.

The above arrangement would be ideal if Alberta-grown Netted Gems were always of top table quality. In these latitudes the variety is medium to late in season and therefore does not always attain top quality in mealiness. Its

greatest fault, however, in this province at least, appears to be the pronounced bitterness which the tubers often develop at sometime during their growth and storage.

It is well-known that sunburn or greening will cause potatoes to develop a bitter flavor. Blodgett and Rich (1949) (6), in a complete survey of potato abnormalities in the Pacific Northwest where the Netted Gem variety is a leader, make one reference to bitterness in the variety; i.e., "where visible greening has occurred from exposure to natural or artificial light of tubers in the field." In stressing its importance, they say only a trace of greening is sufficient cause for rejection of affected tubers as culls, since the whole tuber carries the bitter flavor. Numerous other workers also mention bitterness in potatoes in association with greening (30, 35).

The problem in Alberta, however, is more complicated by the fact that extreme bitterness is found in Netted Gem tubers showing absolutely no sign of visible greening.

Sometimes, accompanying the complaints received concerning bitter potatoes, are reports of sore throats or sickness after eating such potatoes. Experience gained in this study confirms the findings of many of the German workers that such bitterness in the potato is due to an abnormally high solanine content (9, 42, 47).

## Solanaceous Alkaloids (23)

The potato (Solanum tuberosum) belongs to the family Solanaceae. It is interesting to note that the Solanum genus supplies the name for the family as a whole. The significance of this is not known, but the family is noted for the variety of alkaloids found in its various species. It is not surprising, then, that the potato contains an alkaloid-like substance solanine. Other alkaloids found in members of the Solanaceae family are nicotine from Nicotiana spp., atropine from the deadly night-shade Atropa belladonna, "daturine"* from Datura stramonium, the Jimson weed. The belladonna dilates the pupils of the eyes, was formerly used by women for that purpose and so derived its name. "Daturine" is effective as "knock-out" drops, and was formerly used by thieves to stupify their victims and by magicians to produce fantastic visions. Other members of the family containing notably poisonous alkaloids are Black Henbane (Hyoscyamous niger), Bittersweet or Woody Nightshade (Solanum dulcamara), and Black Nightshade (Solanum nigrum). One might speculate that the common name for the family, Nightshade, had its origin in the mystery and superstition associated with the witchcraft that was practised under cover of darkness by medicine men, alchemists and sorcerers who used the alkaloids of the various members of the family to work their charms.

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* Actually hyoscine and hyoscyamine chiefly (23)

## Definition, Occurrence and Metabolism of Alkaloids

As indicated above, the plant alkaloids include many substances which are physiologically active in higher animals -- substances which we know as drugs such as morphine, and quinine, and mildly active stimulants such as nicotine and caffeine (8).

Alkaloids are complex cyclic compounds containing nitrogen that are produced only in certain species of plants. They are especially common in members of the Solanaceae, Papaveraceae, Leguminosae, Ranunculaceae, Rubiaceae and Apocynaceae. Species of plants which contain one alkaloid are very likely to contain others. Most alkaloids are white solids, and as the name indicates, all are basic in reaction (19, 36). In fact, the term alkaloid literally means alkali-resembling substance (37), since compounds of this type have properties similar to those of the alkalies in being able to form, with acids, salts that are usually soluble in water. Miller (37) states that alkaloids may occur in the cell sap of the young parenchyma, or may be stored in older tissue in the leaves, stems and roots.

According to Bonner (8), it has been demonstrated by reciprocal grafts of tomato top on tobacco root and tobacco top on tomato root that the alkaloid nicotine is synthesized in the root of the tobacco plant. From there the alkaloid

appears to be transported through the xylem to the leaves, where it sometimes constitutes up to 8% of the dry weight.

The physiological role of the alkaloids in plants, if any, is unknown (8, 36). Some believe they arise in the formation of proteins, while others consider they result from the disintegration of proteins (37). Whether they function actively in nitrogen metabolism in the species in which they are found, or whether they are only metabolism by-products is not known. The latter role is the most commonly accepted one (36). In any event, their synthesis is dependent directly or indirectly on the photosynthetic mechanism. Miller (37) cites Emde as having stated that photosynthetic activity of plants in general decreases as one progresses from equator to poles, and that alkaloid production of plants decreases concurrently, so that the maximum of plant alkaloids is produced in the tropics while plants of polar regions produce none.

The alkaloids are usually found in plant tissues in the form of salts of organic acids; e.g., malic, citric, succinic, oxalic, tannic, etc. (19, 36). The alkaloid solanidine, however, is found in the potato tuber combined with three sugars to form the glycosidal alkaloid solanine (19, 23, 45, 55).

According to Brautlecht and Getchell (10), the tuber juice is acid. They cite Robertson and Smith as having observed a pH range for potato juice of 5.5 to 6.2 for varieties

grown in the British Isles. Solanine would probably be soluble at this pH.

## Glycosides

From the foregoing account of the occurrence of solanidine, it can be seen that solanine may also be classed as a glycoside; i.e., a compound formed by the reaction between one or more sugars and one or more compounds which are not sugars. Glycosides probably occur more widely in plants than do alkaloids, but like the latter are never present in large quantities. They also resemble alkaloids in many other respects: they are crystalline, colorless and bitter; their role in the metabolism of plants, if any, is also obscure. They are usually soluble in water or alcohol (36).

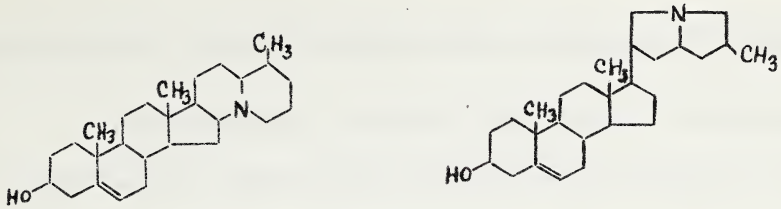
## Solanine, Solanidine and Solanthrene

A knowledge of the chemistry of solanine is important in order that intelligent analytical work be carried out. In the past, there was much disagreement as to the chemical formula of solanine. Henry (23) has compiled seven alternative formulae with nearly as many different melting points proposed by the earlier workers in the field. There is now fairly unanimous agreement as to the molecular formula, but differences in classification still remain. In various texts and papers,

it is referred to as a glucoside, glycoside, gluco-alkaloid or glycosidal alkaloid. These are differences in name only.

The complex is unaffected by alkalies (26), even when boiling (5), but when warmed with acids it is hydrolyzed into the base solanidine and one molecule each of dextrose, rhamnose, and galactose (19, 23, 55). In fact, Soltys and Wallenfels (47), in Germany, report that the acid hydrolysis of solanine not only results in solanidine but some of the solanidine is itself dehydrated to form another alkaloid solanthrene, with the result that solanidine and solanthrene are formed in the ratio of three to one. These workers also state that Bergel and Wagner showed that solanidine consists of one (see Fig. 1), and solanthrene consists of two, double bonds. Wolf and Duggar (55) also confirmed the presence of solanthrene in the hydrolysis products of solanine. They, too, believed it to result from the dehydration of solanidine.

The empirical formula of solanine is now regarded as being $C_{45}H_{73}O_{15}N$ (45), and that of solanidine $C_{27}H_{43}ON$ (14, 19, 45). Competent authorities (19, 55), on the best available evidence, believe that solanidine has one or other of structures illustrated in Fig. 1. Fig. 1 (a) is that proposed by Soltys and Wallenfels, while Fig. 1 (b) is that proposed by Clemo et al. It is evident that the structure of the nitrogen-containing portion of the molecule is still in doubt.




(a) (b)

Figure 1.

Two suggested structural formulae for solanidine: (a) by Soltys and Wallenfels, (b) by Clemo et al. After Gortner (19) .

Solanine is insoluble in water (5), or practically so, and is insoluble in ether, chloroform, benzene and petroleum spirit (5, 23). It is only slightly soluble in cold ethyl alcohol (23); perhaps somewhat more soluble in hot alcohol (5, 23). Either hot ethyl (23) or amyl alcohol (5) may be used to extract solanine from plant tissues, or to dissolve the pure crystals. It also dissolves readily in most acids, including nitric (23), sulphuric (23, 42), acetic (15, 42), and citric (32). Its basic properties are so weak that it is scarcely alkaline to litmus (23). Solanine does not reduce Fehling's solution (45), but will reduce certain other copper compounds (15) and sodium thiosulphate solutions (45). These reducing properties were used by Rooke et al (45) and Conner (15) in hydrolytic methods of determination of solanine in potatoes.

## Physiological and Pharmacological Action of Solanine

The following extract from Allens Commercial Organic Analysis (5) very aptly describes the taste of solanine and some of its reported effects:

"Solanine is odorless when dry, but when moist exhales an odour recalling that of potatoes while cooking. The taste of solanine is somewhat bitter and pungent. It leaves in the pharynx a persistently acrid sensation. Solanine is poisonous, producing in dogs and cats violent vomiting, followed by somnolence and sometimes accompanied by paralysis of the lumbar muscles. One grain killed a rabbit in six hours, and a $\frac{1}{4}$-grain is strongly nauseating to a man. Solanine is stated by Sardas to be an excellent neurotic sedative ........"

Also, quoting Henry (23), in the Plant Alkaloids:

"The solanines are stated to resemble the saponins in action and are described as protoplasmic poisons and potent hemolytics. Their effects are met with only in cases of poisoning by potatoes, which from faulty cultivation or storage or some other cause contain more than usual traces of solanine, but in quite a number of cases of mass potato poisoning the symptoms, while severe, have not proved fatal. The symptoms of solanine poisoning are headache, nausea, emesis (vomiting) and gastritis. With sufficiently large doses parenchymatous nephritis and haemoglobinurea, and even central nervous paralysis

and cardiac arrest, may ensue. It is also a local irritant."

Lepper (32) recommends adsorption on animal charcoal as a suitable treatment for solanine poisoning. Rochelmeyer (44) states that while solanine itself has a stimulating effect, solanidine is regarded as responsible for the toxic effect.

## Normal and Abnormal Solanine Content in Potatoes

Gortner (19) states that potatoes normally contain about 0.024 part of solanine per 1000 (i.e., 2.4 mg. per 100 gm.). Bomer and Mattis (7) found normal potatoes to contain 2.0 to 8.9 mg. %* solanine. They suggest that 20 mg. per cent is the upper limit of safety for food, and that 27.7 to 58.3 mg. per cent caused poisoning. That these latter figures lie within the range sufficient to cause poisoning was confirmed by Harris and Cockburn (21), who found a solanine content of 41 mg. per cent in a sample of potato believed to have caused the death of a five-year old boy in Glasgow. Postmortem examination showed death to be due to strangulation of the bowel, which might have been caused by extreme retching or vomiting. Alfa and Heyl (2) also report outbreaks of illness traced to use of potatoes all grown in one district, the tubers showing a solanine content of approximately 10 to 40 mg. per cent.

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* Mg. % (per cent) means mg. per 100 gm. fresh weight unless otherwise stated.

Table II gives, in chronological order, a summary of the amounts of solanine in mg. per cent reported by various investigators.

Table II. Reported Solanine Concentrations Found in Potatoes

| Date | Workers | Normal | Abnormal |
|---|---|---|---|
| 1907 | Morgenstern (38) | 12.5 | |
| 1918 | Harris & Cockburn (21) | | 41 |
| 1923 | Alfa and Heyl (2) | | 10-40 |
| 1924 | Bomer & Mattis (7) | 2-8.9 | 27.7-58.3 |
| 1937 | Mayfield et al (35) | 8-57 | |
| 1937 | Pfankuch (42) | 3-7 | 20-50 |
| 1943 | Lampitt et al (29) | 7.5 | |
| 1946 | Wolf and Duggar (55) | 1.8-13 | |

As will be seen later, the usefulness of some of these figures lies in their relative rather than their absolute values, since it now appears that the earlier methods of extraction and estimation often led to inaccuracies. The results of Mayfield et al (35) are the only ones which are open to serious question.

## Varietal Differences

Morgenstern (38) analyzed 12 table varieties and found an average solanine content of 12.5 mg. per cent, while seven stock-feeding varieties averaged only 5.8 mg. per cent. He found that "yellow" varieties contained less solanine than "red" or "blue" varieties. Thirteen samples of "yellow" tubers averaged 7.8 mg. per cent solanine, whereas eight samples of "red" tubers averaged 11.9 mg. per cent. "It seems," wrote Morgenstern, "that the higher amounts of solanine in table potatoes may contribute to their flavor as the fodder varieties are lower in solanine but flat in flavor." Dominion Potato Inspector, Mr. J. W. Marritt, of Edmonton, Alberta, expressed the same idea to the author in conversation. He believes that certain amounts of solanine may be necessary for quality of flavor in the table potato.

Fellenberg (17) reported the solanine content of "Yellow Mouse" and "Danish" potatoes were 2.6 mg. per cent and 5.7 mg. per cent, respectively. Lampitt (29) found that the amount of solanine in sprouted seed potatoes varies with the variety, and that free solanidine occurs in the sprouts of some varieties but not in others.

Wolf and Duggar (55), in a survey of the solanine content of 32 potato varieties grown in the United States, also found evidence that solanine content varies with the variety

(see Table III). They state, further, that analysis for solanine content of varieties is of little comparative value unless tuber size is taken into account, because within a variety they found that total solanine content increases with the tuber size (at least up to 135 gm.) but solanine concentration decreased. In this connection, Braun (9) reports that samples of the same variety often give different values, but that high solanine content was often associated with certain varieties, especially with the variety "Ovalgelbe."

Some of the varietal results obtained by Wolf and Duggar (55) are reproduced below in Table III. Unfortunately, samples were not drawn from one locality but from two.

Table III. Varietal Differences in Solanine Content of Potatoes. After Wolf and Duggar (55)

| Variety | Average solanine content in mg. per 100 gms. fresh tuber | Time of Maturation |
|---|---|---|
| Columbia Russet | 1.8 | Late |
| Russet Rural | 2.1 | do |
| Sebago | 2.1 | do |
| Triumph | 3.2 | Early |
| Earlaine | 3.9 | Midseason |
| Green Mountain | 4.0 | Late |
| Wee McGregor | 4.2 | Midseason |
| Russet Burbank | 4.7 | Late |

Table III (cont'd)

| Variety | Average solanine content in mg. per 100 gms. fresh tuber | Time of Maturation |
|---|---|---|
| Early Ohio | 4.8 | Early |
| Katahdin | 5.5 | Late |
| Irish Cobbler | 5.6 | Early |
| Chippewa | 6.6 | Midseason |
| Warba | 6.9 | Early |
| Early Epicure | 8.9 | do |
| Pioneer Rural | 10.0 | Late |
| Hindenburg | 11.6 | do |
| White Rural | 13.0 | do |

## Factors of the Cultural Environment

### Soils, Fertilizers and Moisture

Morgenstern (38) reported in 1907 that humus, moisture and potash diminished the solanine content of the tubers, but that dry sandy soils, phosphoric acid, and especially nitrogen, increased their solanine content. On the other hand, Pallman and Schindler (41) found in 1942 that fertilizing, particularly with high dosages of nitrogen and potassium, had no effect except on the solanine content of young tubers. Other workers (7, 9, 32) report that fertilizers have no effect.

The above evidence is contradictory, probably because none of the nutrient levels were determined before the fertilizers were applied.

Minor elements also play an important part in the potato plant metabolism. Brautlecht and Getchell (10) believe that magnesium plays a vital role in the carbohydrate metabolism of the potato. They report that it is found more abundantly in potatoes than is calcium, and soils are depleted of it more rapidly. The tops, leaves and vines, contain about five times as much magnesium as the tubers. The high concentration in the potato leaves is associated with the chlorophyll. It is suggested that the magnesium may function in the formation of the carbohydrates. It is further suggested that sunburned potatoes, with a green skin and a green color often extending to a considerable extent into the potato, may possibly involve a lack of conversion of chlorophyll to starch or a change from starch to sugar to chlorophyll.

If Brautlecht and Getchell are correct in their assumptions, it seems logical that adequate amounts of available magnesium may be a necessary prerequisite to normal metabolism of solanine in the potato, and that abnormal chlorophyll formation in the tuber is in all likelihood accompanied by or substituted with solanine formation.

That mineral deficiencies do not necessarily exhibit external symptoms was shown by Hayman (22) who reports increased yields of potatoes grown in the Red River Valley by applying zinc-containing dusts and sprays to vines that showed no external symptoms of zinc deficiency.

## Date of Planting and Maturity

Unripe tubers are generally reported to have a higher solanine content than mature ones (32, 38). Wolf and Duggar (55), however, presented evidence (see Table III) to show that there is no obvious correlation between time of maturing and solanine content. Small tubers are usually reported higher in solanine than large ones (7, 32, 38). Morgenstern (38) reasoned that this difference is due to the greater peel area per total volume. The tendency reported by Clark (13) for the smaller tubers to be found on the upper stolons may also be a factor. Braun (9) reports that no correlation between solanine content and planting date could be demonstrated.

## Climate

According to Arutyunyan (4), the solanine content of Armenian potatoes amounts to 0.010 to 0.014 per cent of dry weight depending upon climatic conditions. He maintains that the solanine content of potatoes grown in mountainous sections

with temperate climate is always less than that of those grown in hot climates. In the latter case, summer cultivation of the potato field is recommended to reduce solanine content.

Environment or Heredity?

Gortner (19) claims that the alkaloidal content of plants may be increased or decreased by appropriate selection of strains, by plant breeding and by fertilization (especially with nitrogen and phosphorus). He cites the nicotine content of tobacco as an instance of an alkaloid that has been increased by plant breeding and manuring. Loustalot and Winters (33) found that the roots and stems of plants of Cinchona ledgeriana grown at high nitrogen level contained higher amounts of total alkaloid than did those plants grown at lower nitrogen levels, while total alkaloid and quinine sulphate in roots of seedlings grown in medium and high soil moisture were significantly higher than those in roots of seedlings grown in low soil moisture (34). Evidently, then, in certain instances environment plays the greater role. Solanine-rich potatoes as seed have been found to produce tubers of normal solanine content (7, 53), though in some cases it took two seasons for the proportion to reach normal (29).

There seems reason to believe, on the other hand, that the solanine content of potatoes may be reduced by breeding

and subsequent selection to a point where no further trouble would occur with poisoning or bitterness.

## Light and Photoperiod Effect on Plants and Tubers

Wolf and Duggar (55) at the University of Wisconsin, in a study of solanine synthesis and distribution, found that "in the growing shoot with adequate supply of carbohydrates the synthesis of solanine is apparently independent of light, although light affects the distribution of the glycoside. In etiolated plants, there is a fairly uniform distribution between tops and roots, whereas in light a larger proportion remains in the tops. The photoperiod influences both the distribution and the synthesis of solanine. Plants about ten weeks old display a higher content of solanine under a day-length of eighteen hours than plants of similar age under a ten-hour day. Furthermore, the alkaloid concentrates in the tops of long-day plants. In general, the data obtained confirmed the view that solanine occurs commonly in highest concentration in those organs and tissues where metabolic activity is greatest." Lampitt et al (29), working in England in 1943, reached the same conclusion and stated it thus: "The very large amounts of solanine found in the sprouts of potatoes is perhaps most interesting as an indication of the metabolic changes which take place when the resting stage gives place to the active growing stage."

The solanine content increases when potatoes are exposed to daylight (38), sunshine (19, 32) or ultra-violet light (4, 15). Lepper (32) found the highest solanine content (55.7 mg. per cent) in the small green tubers of the Voran variety. Irradiation by sunlight increased slightly the solanine content in a strongly green potato, but storage reduced the increased solanine content obtained by irradiation. Sunburned potatoes are known to have as high as 58.8 mg. per cent solanine (19). Bomer and Mattis (7) reported no increase in solanine content in old potatoes after exposure to light for 7 - 10 weeks, while young potatoes with and without tops attached developed large amounts of solanine on exposure to light. This seemed to be related to formation of green color, which occurs much more readily on immature or "young-mature" than on "old-mature" tubers, when they are exposed to light.

In experiments with the effect on the potato tuber of different wave-lengths of light, Conner (15) found that rays from the blue end of the spectrum (ultra-violet rays of about 0.3 $\mu$) were most effective for solanine formation but did not cause greening; whereas the yellow-red end of the spectrum was efficient for glucose synthesis, caused greening, but did not result in significant increases in solanine.

## Disease and Injury

Forty-three years ago, Morgenstern (38) reported

that diseases such as rust, soft rots and rhizoctonia do not affect the solanine content of tubers. However, some potato diseases, such as those caused by viruses, probably were not recognized at that time. No recent information is available on the effects of viruses or other potato diseases on the amounts of solanine present. It is possible that solanine bitterness may be a non-parasitic physiological disease or disorder itself, to which the variety Netted Gem is highly susceptible. In fact, the susceptibility of Netted Gems to solanine bitterness in Alberta closely parallels that of the variety Ontario for internal necrosis under Wisconsin conditions. Larsen and Albert (31) for five years studied at digging time the incidence of internal necrosis, a non-parasitic physiological disorder. They found that varieties differ in their susceptibility. Some are entirely free, some show it in an intermediate degree, others are highly susceptible to it. The variety Ontario is the only one which develops it on muck soils in Wisconsin. It is noteworthy that in the 1949 season Ontario was the only variety which exhibited extreme susceptibility over a wide range of conditions and soil types in Wisconsin.

Morgenstern (38) noted that potatoes damaged in the soil had slightly more solanine than undamaged tubers in the soil. This phenomenon suggests the idea that damage to the tops by hail or frost might cause increases in the solanine of the tuber.

## Factors of the Storage Environment

While the present investigation is primarily concerned with the effect of field cultural practices, a study of the reaction of the potato to the storage environment may throw some light on its reaction to the field environment. Furthermore, the amount of solanine in the stored tuber is the sum of these two reactions.

Morgenstern (38) reports that the solanine content of tubers is unaffected by long storage, while 30 years later Naumov (39) of the U.S.S.R. reports increases in storage at low temperatures but a decrease if stored at $5^{\circ}$ - $7^{\circ}$ C ($40^{\circ}$ - $44^{\circ}$ F approx.), the decrease amounting to 50 per cent if the tubers are washed before storage. Wolf and Duggar (55) report that storage of the tubers at $4^{\circ}$ - $5^{\circ}$ C for long periods after harvest does not appreciably affect the solanine content of the varieties they tested, but analyses should be made within six or seven months because a slight rise is observed beginning in April or May. They state , further, that when solanine content is determined on a fresh weight basis, any water loss from the tuber leads to an apparent increase in solanine. Morgenstern was more accurate when he attributed the apparent increase to loss of water and carbon dioxide because of respiration and transpiration.

In this connection, the work of Treadway *et al.* (52) is most interesting. They studied the effect of storage

at 34° to 60° F on composition. They found that the loss of carbohydrate material and of moisture occurs at about the same rate so that the percentage solids remains at the original level. They cite Appleman as having found that three processes occur in the potato: (a) respiration which consumes sugar by converting it into carbon dioxide and water; (b) conversion of starch to sugar by amylolytic enzymes; and (c) conversion of sugar to starch (presumably by starch synthesizing enzymes). At low temperatures sugars increase and starch decreases; at higher temperatures sugars decrease as a result of respiration and starch synthesis.

Alexander et al (1) encountered bitterness in the flavor of potato chips made from Katahdin variety, kept under low temperature storage conditions from October to February and March. Along with this bitter flavor were flavors described as sweet and burned by the judges. All were associated with excessive accumulation of reducing sugars. As progressive conditioning lowered the sugar content, the sweet and burned flavors disappeared, but the bitterness persisted in many lots. There were, however, fewer reports of bitterness among the french fries made from the mealy potatoes than from the less mealy. Conditioning was done by storing at 70° - 75°F for periods varying from one to ninety-one days. Reducing sugars were estimated by a picric acid coloration method.

The influence of light has been dealt with, in part, under cultural environment. It seems reasonable to believe that there is an interaction between the factors of light, temperature and humidity of storage, as well as varietal inheritance. This problem will be dealt with under the next heading.

Development of Greening and Acrid Flavor in Potatoes

Mayfield et al (35), in studying the effect of winter storage on palatability and nutritive value of potatoes, noted that a greenish-yellow color developed in the flesh of Netted Gem potatoes when kept in a cool, damp, dark cellar ($37^{\circ}$- $46^{\circ}$ F), and that this change in color was accompanied by an acrid flavor. The change was noted in January and became more marked as storage advanced. These observations were made over a period of seven years. The Bliss Triumph stored during three seasons in the same cellar varied in flesh color change from year to year, sometimes remaining white and at other times becoming greenish-yellow, but with this change in color did not develop an acrid flavor.

In a warm, dry storage cellar kept at $55^{\circ}$ - $60^{\circ}$ F with subdued light (another part of the same experiment), neither Netted Gem nor Bliss Triumph potatoes showed any indication of developing an acrid flavor. Although they withered and sprouted, their tissue retained its normal color and normal potato flavor.

Regarding light, etc., Mayfield says that in the cool and damp storage the temperature, humidity, light and ventilation were under control. The warm, dry storage was described as typical home basement storage, with light and ventilation supplied by a north basement window and no attempt made to control temperature or humidity. The writer's interpretation is that the cool, damp storage had <u>no light</u>, and the warm, dry storage had <u>subdued light</u>.

Mayfield and her co-workers, already cited, made an analysis for solanine content periodically in the two varieties. Solanine content was found to be about the same for each variety. It was lowest a month before harvest (1.4 - 8 mg. per cent), and highest on May 1st after seven months' storage (51 - 57 mg. per cent). It was found that though Netted Gem tuber flesh developed much more color and acrid flavor than that of Bliss Triumph variety, it contained no more solanine. Thus it would appear that tuber flesh color and even acrid flavor may not be connected directly with solanine content.

However, since Mayfield's figures for solanine content are so far out of line with those usually quoted for normal potatoes (see Table II), it may well be that not much confidence can be placed in her conclusions. A gravimetric method of analysis was used, and it seems doubtful if pure solanine was measured.

Mayfield's maximum percentages appear to be dangerously high (cf. the upper limit for safety for food, set at 20 mg. per cent by Bomer and Mattis), but she reports feeding rather large amounts of potatoes where the solanine content was relatively high to rats and guinea pigs in the course of vitamin tests without any toxic symptoms attributable to solanine.

In this connection, Hansen (20), after chemical and biological studies, came to the conclusion that "alleged solanine poisoning cannot be attributed to solanine, but to some form of bacterial toxin. The evidence leading to this conclusion is that in the gastro-intestinal tract solanine undergoes hydrolysis with the formation of solanidine, which is practically insoluble and consequently not absorbed into the blood stream."

It should be pointed out that solanidine is a sterol derivative, and though sterols generally are insoluble in aqueous solutions, one of them, at least, (cholesterol) is found in quantity in the blood stream. Schoenheimer (46) has shown that cholesterol is a sterol of animal origin; is synthesized in the animal body; and also may be absorbed as an ester reaching the blood stream via the thoracic lymph. There is the possibility that other sterols or their derivatives may be absorbed from the intestinal tract, as esters of fatty acids. Certainly, cholesterol is soluble in bile, which is some reason to believe that solanidine is also soluble in bile and may be absorbed as a fatty acid ester into the blood stream. However,

Hansen's conclusions that solanidine is not absorbed in the intestinal tract seems to be verified by Schoenheimer (46), who has presented very convincing evidence that plant sterols are not absorbed as esters in the thoracic lymph duct. Schoenheimer admits that his experiments involved the use of chemical methods which might introduce slight experimental errors. For this reason, he could not rule out the possibility that there might have been absorbed the minutest traces of plant sterols which were not detectable by the methods used.

Working on what he termed "greening in potatoes," and which he differentiated from "sunburn," Larsen (30), of Idaho, attributed greening to chlorophyll formation but did not correlate it with palatability or solanine content. Chlorophyll formation was shown to be closely correlated with visible greening, and green and blue screens were found effective in reducing it. Little or no greening was found to take place below 40° F. Tubers of the variety Netted Gem were found to develop slightly but significantly less chlorophyll upon exposure to light than did tubers of the variety White Rose. The maturity of tubers at harvest, and exposure to sunlight immediately after digging, had no effect on the amount of greening taking place upon subsequent exposure to artificial light. Most rapid formation of chlorophyll occurred at the highest temperature tested (68° F) at the light intensity used (9 foot-candles).

In 1950, the author (27) conducted some preliminary storage trials with Netted Gem potatoes which included three storage temperatures without light and some with subdued light. Degree of bitterness was tested by tasting the pressure-cooked tubers. It was not found that greening appreciably increased the bitterness, but it was found that greening produced of itself another off-flavor distinct from the bitterness ascribed to solanine. Hilton (24) concluded from the data on these trials that, in general, low temperature storage maintained or caused more bitterness in the variety than storage temperatures at $10^{\circ}$ C or above.

The evidence, therefore, regarding the effect of temperature on solanine bitterness seems to be: (a) that the higher storage temperatures are likely to be least favorable for the development of bitter or acrid flavor, and, therefore, possibly solanine; and (b) that subdued winter daylight of low intensity may not favor its development either. These conclusions seem to be supported in whole or in part by the reports of Naumov (39), Mayfield (35) and Hilton (24). The apparent increased bitterness in certain varieties of potatoes stored at low temperatures may be due, not to any greater synthesis of solanine, but to the splitting-off of the reducing sugars - glucose, galactose and rhamnose, leaving the alkaloid solanidine, which may give a greater apparent bitterness to the flavor.

## Distribution of Solanine in the Potato Tuber

The proportion of solanine present in the eyes of the potato is greater than that in the skin, which, in turn, is greater than that in the flesh (18, 19, 29). Sprouts contain much greater quantities than either peel or eyes (28, 38), and it may be significant that sprouts contain notable quantities of solanidine (29). Fischer and Thiele (18), using a blood-gelatin test, found solanine principally in the first ten cells of storage tissue.

Summing up their findings in England, Lampitt *et al.* (29) report: "Apparently, during storage, and presumably as a first stage in sprouting, the solanine content of the eyes increases, and then as sprouts develop, decreases, while that of the flesh remains unchanged throughout. The solanine and solanidine content of the peel and flesh decrease when potatoes develop sprouts in the dark, at both 30° C and 4° C, but little change occurs at room temperature in daylight. In the dark, at 4° C (approx. 38° F), where little or no sprouting was evident, a decrease in the solanine content of the whole tuber occurs, whereas under other conditions of storage described the total solanine increases."

## Distribution of Solanine in the Entire Potato Plant

Table IV shows the distribution of solanine in the parts of the potato tubers and plants as found by Lampitt

et al (29).

Table IV. Solanine Content of Various Parts of Potato Tubers and Plants. (After Lampitt et al.)

| Part or Plant of Tuber | Solanine in mg. per cent (fresh weight basis) |
|---|---|
| Skin (2% - 3% of tubers) | 30 - 64 |
| Peel (10% - 12% of tubers) | 15 |
| Peel incl. eye (1/8 in. disc) | 30 |
| Peel excl. eye (1/8 in. disc) | 19 |
| Flesh | 1.2 - 10 |
| Whole Potato | 7.5 |
| Sprouts formed during irradiation of tuber | 420 - 730 |
| Flower | 215 - 415 |
| Leaf | 55 - 60 |
| Stem of haulm | 2.3 - 3.3 |

Evidently, the per cent solanine is greater in the sprouts, leaves and flowers than in the whole potato. Street et al.(49) found the concentration in the roots in May and June very high (68 mg. per cent), but it fell during the growing season to 1 mg. per cent by August 22.

## The Effect of Cultural Practices on Yields

### Tuber-Bearing Parts, Tuber Formation and Rate of Growth

An understanding of how tuber formation occurs is useful in a study of this nature for two reasons. It is an aid in interpreting yield differences from cultural treatments, and it is a guide in taking field samples for solanine analyses. Clark (13) gives an excellent description of the tuber-bearing parts.

The tubers are borne on underground stems, commonly called stolons, which are modified branches arising from nodes of the main stem, and are normally situated below ground. The development of the buds into stolons rather than leaf-bearing branches depends largely upon certain definite environmental conditions, chief among which is the exclusion of light. According to De Vries quoted by Clark (13), each axillary bud of the stem of a potato plant has the capability of developing, according to the conditions which surround it, into either a leafy stem or a tuber-bearing stolon. Clark states, further, that this was substantiated by Franz, who claims that if the lower buds are exposed to light, green shoots will generally develop instead of stolons; likewise, the upper axillary buds will develop into stolons instead of green shoots, if light is excluded early enough.

The arrangement of stolons on the stalk is generally five-ranked, though there are many exceptions to this which are attributed to the failure of some nodes to develop. This same arrangement occurs with the leaf scales on the stolons. The stolons terminate with leaf scales which are strongly recurved, usually at an angle between 90° and 180°.

The stolons start comparatively early in the growth of the plant, usually within a week or ten days after the plants have appeared above ground, the stolons of the early varieties appearing a little in advance of those of the late ones. Under normal conditions of growth, the stolons should be well started by the time the plants have reached a height of four inches.

The length of the stolons is subject to great variation, ranging from less than an inch to lengths equalling, or occasionally exceeding, 18 inches. While there is much variation within the variety, and, in fact, the individual plant, there is a fairly well-defined relationship between stolon length and variety. Stolon length is greater under conditions of long days, while tuberization is delayed.

Clark (13) studied tuber formation and rate of growth under irrigation for two years with the late variety Rural New Yorker at Greely, Colorado. He reports that practically the entire crop of tubers was set at the beginning of the

period of tuber development, probably within the space of a very few days. The time of beginning of the tuber formation was found to coincide very closely with the end of the period of flower-bud development.

The rate of growth was found to be very rapid during the early part of the period of tuber development. Maximum rate of growth of tubers was found to occur about the last of August or first of September, which was approximately 80 days after planting, at which time nearly one-third of the total period of tuber development had elapsed. In both years, there was a progressive increase in weight per hill and in average weight per tuber throughout the entire period of the investigation. Differences in the sizes of tubers in the individual hill were attributed largely to an unequal rate of growth, rather than to differences in the age of the tuber. The weight of tuber did not appear to be correlated with the length of stolon on which it is produced, but there was a tendency toward a decrease in size of tuber on the upper stolons. While Clark (13) found that a small increase in the weight of tubers occurred after the vines had been killed by frost; he cites Follstad as having obtained results that showed an average gain in weight 17 days after the death of the foliage of 26.4 per cent or 57 bushels per acre under Wisconsin conditions.

Tuber Yield as Affected by -

(1) Number of Eyes in Seed-Pieces

In studying the influence of the number of eyes in the seed set upon the yield of potatoes, the U.S.D.A. publication from the Office of Horticultural Investigations by Stuart et al (50) is of special interest. Although this publication is twenty-eight years old, the literature review extends back to 1776; that is, almost another 150 years. In 24 out of 32 reports reviewed in which single eyes were used, in comparison with multiple-eye sets, the results from the multiple-eye sets were better.

Judging from the context of most of the reports, the lower yields from the single eyes was attributable, in part at least, to the smaller piece of tuber that may be associated with the single eye. To cite a specific example, Anderson in 1776 as quoted by Stuart et al.(50) reported one-eye sets from the apical and basal portions of the tubers showed an increase of more than 123 per cent in favor of the basal eye sets. As more one-eye sets can be made from the apical end than from the basal end, presumably the one-eye sets from the apical portion of the tubers were smaller than those from the basal portion, for one of Anderson's conclusions was that the weight of the crop is always in some measure influenced by the weight of the seeds planted. This principle was clearly illustrated in his results, where large whole tubers (average weight 6.5 oz.) from which all

but one eye had been removed outyielded the one-eye sets (average weight 0.13 oz.) from the basal parts of large tubers.

The above results were confirmed by Zavitz, who is also quoted by Stuart et al (50). Zavitz, in a 5-year test, showed that increasing the weight of single-eye seed-pieces from 1/16 oz. to 2 oz. gave progressive yield increases from 46.2 to 132.7 bushels per acre, even after seed used was deducted. But he also found that for a given seed size increasing the number of eyes from two to five gave increased yields. On the other hand, Schollander, as cited by Stuart _et al_.(50) in a summary of a five-year yield study of large and small whole tubers, large halves, and of large three-eye, two-eye and one-eye pieces, reports that large one-eye sets came first in yield of merchantable tubers, and second only to large whole tubers in total yield. It is noteworthy that the large one-eye sets outyielded the large two-eye and three-eye sets on both points.

From the foregoing, there would seem to be fairly general agreement that the size of seed-piece is a factor affecting yield, but there is less agreement as to how much yield is affected by the number of eyes. However, Thompson (51), who also reviews the work of Zavitz and others, concludes that yield is influenced mainly by the size of seed-piece irrespective of the number of eyes, and that therefore the question of number of eyes becomes of only minor importance. Thompson (51) says that

Whipple thinned plots of several varieties in Montana to one stalk per hill and reported a very small increase in marketable yield, with practically no difference in total yield. Thompson (51) concludes, therefore, that the most important factor in cutting seed appears to be that of obtaining a sufficiently large piece to insure at least one vigorous stalk.

Wakankar (54), working in India, studied the effect of size of seed-piece and number of sprouts on the resulting potato crop. He used whole seed tubers 40 grams in weight, but limited the number of sprouts to one, two and three, respectively, in six replicates each, with the tubers planted a foot apart. The results showed that keeping the seed size constant, the yield, total number of tubers produced, and their individual size are affected considerably by the number of sprouts per hill. With one sprout to each hill, the total number of tubers formed and the yield in each hill is less, but the individual size of the tubers produced is increased. On the other hand, when three sprouts are kept to each hill, the total yield and the number of tubers formed is increased, but their relative size is reduced.

However, comparing the yields from seed-pieces with one sprout but differing in weight, i.e. 10, 20, 40 grams, had no significant effect on the total yield, the total number of tubers, and the individual size of the tubers produced.

Wakankar (54) concludes that large seed-pieces give higher yields because of their capacity to produce more sprouts (stems) per hill. Even a single eye may often produce more than one sprout or stem, especially if the seed-piece is large.

(2) <u>Planting Date</u>

Thompson (51) states that, if possible, potatoes should be so planted as to bring the period of blossoming and tuber setting during a time when weather conditions are optimum. This period, he says, is usually about six weeks after normal planting date. He cites the work of Erwin and Rudenick and that of Bushell, all working in Ohio, and the consensus seems to be that normal planting time gives better yields than early or late planting. Rich (43), in a five-year study with Green Mountain potatoes in Rhode Island, reports potatoes planted April 25, May 5, 15 and 25, produced yields and percentages of No. 1 potatoes in descending order.

(3) <u>Depth of Planting and Degree of Hilling</u>

There appears to be a paucity of literature dealing with these factors. However, Thompson (51) covers these factors very neatly in one paragraph, which is quoted here in full:

"Depth of planting varies mainly with soil type and with the system of culture. Although 4 inches is about average, the depth is commonly more shallow on heavy soils and deeper on

light soils. Irrespective of depth planted, tubers tend to develop at the 4-inch depth. This is due to the fact that conditions of soil moisture and temperature are optimum for growth at this depth. On heavier soils, the seed is commonly planted quite shallow, and a system of gradual-to-steep ridging is practiced during the growing season. This provides against injury to the crop from excessive rainfall and makes digging easier. Extreme ridging is practiced chiefly in Maine, western New York, in the South, and in irrigated sections of the West. Elsewhere more nearly level culture is practiced. In regions where soil is light and rainfall is likely to be insufficient, ridging is sometimes overdone with the result that the soil dries out and growth is retarded at midseason."

# EXPERIMENTAL PROCEDURE

## Experimental Design

The study of the effect of field cultural practices upon the occurrence of solanine in the potato tuber appears to be a subject upon which no reliable information is available. Consequently, it was decided to study the influence of a number of possible factors. With this idea in view, two factorial designs were set up side by side, each in three replicates. The experimental unit was a 30-foot row with a guard row on each side receiving the same treatment. Thus each treatment row was separated from another treatment by the two guard rows. Seed-pieces were planted by hand a foot apart in furrows 36 inches apart and plowed under. The rows ran north and south. Replicates numbered also from north to south. Guard hills at each end of the treatment row were discarded to minimize border effects. Though the occurrence of solanine in the tubers under differing cultural practices was the primary objective, concomitant yield results were recorded and assessed.

(1) Experiment to study the effects of normal and late planting, and single and multiple-eye seed-pieces, on tuber yields and solanine content.

The field plot consisted of a split-plot arrangement in which normal and late planting occupied the main plots

at random within each replicate, and randomly arranged were the sub-plots of two types of seed-piece, single and multiple eyes, respectively.

Planting dates were: normal, May 18, 1950, and May 22, 1951; late, June 9, 1950, and June 11, 1951.

(2) Experiment to study the effects of deep and shallow planting, and degrees of hilling, on tuber yields and solanine content.

Again the field plan consisted of a split-plot arrangement. Two depths of planting randomly arranged in each replicate comprised the main plots. The latter were subdivided into three sub-plots of randomly arranged degrees of hilling, designated no hilling, low hilling, and high hilling.

Although the designation, deep planting, was used, deeper than normal planting was not intended. The term "normal planting depth" was not used so as to avoid confusion with "normal planting date" used in the other experiment.

Shallow planting was achieved by raking the soil back into the furrow to a depth of about three inches. The soil was a typical Edmonton clay loam. Plots were rolled with a land packer after planting, and given one shallow cultivation shortly after emergence. Thereafter, weeds were eliminated by scraping the surface with a sharp hoe. Hilling was done by manual labor with the hoe on July 24 and July 25 in 1950, and

on approximately the same dates in 1951. The intent was to hill after tuber formation was assumed to have taken place.

## Preparation of the Seed-pieces

Tubers of the variety Netted Gem were cut into seed-pieces in the normal manner. All seed-pieces were kept fairly uniform in size and averaged about two ounces. Each seed-piece contained two or more eyes except where single-eye pieces were desired, in which case seed size was maintained equal to that of multiple-eye pieces but the number of eyes were reduced to one. No preference or bias was shown toward apical or basal tuber parts for either single or multiple-eye seed-pieces.

Second generation certified Netted Gem seed was used in 1950, and third generation of the same stock was used in 1951. The first year the seed-pieces were treated with fermate. No treatment was applied to the seed the second year. There was a rather high incidence of leaf roll and mosaic the first year, but with careful roguing almost no disease appeared during the second year.

## Methods of Taking Field Samples

### (a) For Yield Data 1950 and 1951

The treatment rows were dug with digging forks

and total yield in pounds taken on the 30-foot row less the guard hills at each end. In 1950, yields were based upon a possible 28 hills, while in 1951 they were based on 27 hills, as 31 seed-pieces were planted in the 30-foot row the second year instead of 30 pieces as in 1950. Yields were recorded to the nearest half-pound in 1950, and to the nearest pound in 1951. Due to the above differences and to the facts that the trials were at two different locations in the two years and that rogueing increased the "missing hill" counts in 1950, a combined analysis of variance was not attempted.

(b) For Solanine Analysis

Before any potatoes were picked up, 20 to 30 tubers ranging in size from approximately 3 ounces upward were taken at random from the dug row, placed in a cotton sack, labelled inside and out and weighed with the remainder of the row. The samples were promptly stored in a cool root cellar the same day as dug.

## Selection and Preparation of Tubers for Solanine Analyses

In 1950, the majority of samples to be analyzed remained in storage until March, when it was decided to quick-freeze representative samples for analysis throughout the spring and summer months. Consequently, six 4-ounce tubers of each

treatment were washed and allowed to dry. They were then quartered with peel intact, put through a food chopper, and minced in a Waring Blendor for not more than two minutes. Quadruplicate samples of 150 grams each were carefully weighed into previously labelled and waxed paper-cup containers, lids put on, quick-frozen and stored until required.

A somewhat better procedure was followed in 1951. The tubers were dug on October 8, and to prevent the cultural treatment effects from being complicated by a long storage history, samples were quick-frozen a month after digging as follows:

The sample taken in the field was emptied out and the tubers grouped according to their respective sizes. The number of tubers selected to make up the sample for analysis was limited by the volume of potato material that could be efficiently minced at one time in the Waring Blendor. As this amount appeared to be the equivalent of three to four tubers, a total of seven tubers was selected at random from the representative size groups. That is, if there were three size groups - small, medium and large - and more tubers appeared in the small group, then selection was made as follows: two large, two medium and three small. These tubers were halved longitudinally, and the one half of each tuber was discarded. The remaining seven halves were prepared as before except that they were minced for five minutes, weighed in quadruplicate 25-gram samples in small numbered aluminum cans, quick-frozen and stored.

## TUBER YIELDS

### Tuber Yields as Affected by Normal and Late Planting with Single and Multiple Eyes

The data for 1950 are presented in Table V according to treatments and replicates, while Table VI summarizes the same results according to treatments only. The split-plot factorial analysis of variance for these data is presented in Table VII. Data for 1951 are similarly presented in Tables VIII, IX and X, respectively.

### Discussion

It has been pointed out that single eyes can produce more than one sprout or stem. In these experiments, then, where seed-pieces were of relatively the same size, their ability to produce optimum number of sprouts would be about the same regardless of the number of eyes in the seed-piece. The result could have been that there was relatively little difference between the number of sprouts developed by some single-eye and some multiple-eye seed-pieces. Although no counts were taken of the number of stems in the field from single and multiple eyes, it was observed that some of the single-eye seed sets produced more than one stem.

It seems possible, therefore, that any yield differences that might theoretically have occurred between single

stems and multiple stems from single and multiple eyes, respectively, were modified in practice by the equal weights of the seed-pieces tending to result in (1) some single eyes producing multiple stems, and (2) some multiple eyes producing single stems.

The only consistent trends for the two years was that planting at normal time, May 18 - 20, gave higher yields than late planting, June 9 - 11. The increased yields due to normal planting date did not prove significant in either of the two years.

## Tuber Yields as Affected by Shallow and Deep Planting with High, Low and No Hilling

The data for 1950 are presented in Table XI according to treatments and replicates, while Table XII summarizes the same results according to treatments only. The split-plot factorial analysis of variance for these data is presented in Table XIII.

### Discussion of 1950 Results

Inspection of the yield results summarized in Table XII shows certain interesting but rather unimportant trends. In what was a relatively dry growing season, neither shallow planting nor high hilling appeared to have a depressing effect on mean yields. In fact, a tendency for the reverse seemed to exist.

Though drying-out of the soil due to extreme ridging would be expected to occur as Thompson (51) says it does where rainfall is light, such a process, if it occurred, was not reflected in lower yields. The trenches left from the ridging may have served as catch basins for the sudden heavy rains that fell late in the season, i.e. in August.

The slightly higher mean yield from the shallow planting (Table XII) cannot be explained on this basis, however. It might be partly explained on the basis of earlier emergence and better capitalization on the early spring moisture at planting time. However, increases due to high hilling over no hilling, and those due to shallow planting over deep planting were very slight, and probably can most safely be attributed to chance.

The split-plot factorial analysis of variance table shows that the treatments studied had no significant effects on yield. (Table XIII.)

## Discussion of 1951 Results

As can be seen by inspection of Table XV, shallow planting again showed a higher yield trend. This variance due to planting depth, though fairly high, was not significant (Table XVI).

In the 1951 season of well-distributed rainfall throughout the growing season, the no-hilling treatment gave

the highest mean yield. Why the low hilling showed a trend for lower yields in both deep and shallow planting cannot be explained, unless this type of hilling tended to cut more feeder roots under the particular conditions of the 1951 season.

Table V. Tuber Yields in 1950 from Normal and Late Planting; Single and Multiple Eyes; Data in Pounds from 28 Hills

| Planting date | Eyes in seed-piece | Replicates I | II | III | Treatment means |
|---|---|---|---|---|---|
| Late | Single | 10.0 | 11.0 | 11.0 | 10.7 |
| | Multiple | 9.0 | 16.0 | 16.5 | 13.8 |
| Normal | Single | 12.5 | 20.5 | 12.5 | 15.2 |
| | Multiple | 18.5 | 20.0 | 18.0 | 18.8 |
| | Replicate means | 12.5 | 16.9 | 14.5 | 14.6 |

Table VI. Summary of Treatment Means from Table V

| Planting date | Single eyes | Multiple eyes | Means |
|---|---|---|---|
| Late | 10.7 | 15.2 | 12.3 |
| Normal | 13.8 | 18.8 | 17.0 |
| Means | 12.6 | 16.0 | 14.6 |

Table VII. Analysis of Variance of Potato Yield Data from Table V: Normal and Late Planting; Single and Multiple Eyes 1950

| Variance due to - | DF | S.Sq. | M.Sq. | F | 5% | 1% |
|---|---|---|---|---|---|---|
| Replicates | 2 | 38.37 | 19.19 | 2.78 | 19.0 | |
| Dates | 1 | 67.69 | 67.69 | 8.39 | 18.51 | |
| Main plot error (1) | 2 | 16.13 | 8.07 | | | |
| Eyes | 1 | 35.01 | 35.01 | 5.35 | 7.71 | |
| Eyes x dates | 1 | 0.20 | .20 | | | |
| Error (2) sub-plot | 4 | 26.16 | 6.54 | | | |
| Total | 11 | 183.56 | | | | |

Table VIII. Tuber Yields in 1951 from Normal and Late Planting; Single and Multiple Eyes; Data in Pounds from 27 Hills

| Planting date | Eyes in seed-piece | Replicates I | II | III | Treatment means |
|---|---|---|---|---|---|
| Late | Single | 48 | 50 | 52 | 50.0 |
| | Multiple | 62 | 43 | 42 | 49.0 |
| Normal | Single | 62 | 63 | 60 | 61.7 |
| | Multiple | 57 | 62 | 58 | 59.0 |
| Replicate means | | 57.3 | 54.5 | 53.0 | 54.9 |

Table IX. Summary of Treatment Means from Table VIII

| Planting date | Eyes in seed-piece Single eyes | Multiple eyes | Means |
|---|---|---|---|
| Late | 50.0 | 49.0 | 49.5 |
| Normal | 61.7 | 59.0 | 60.3 |
| Means | 55.8 | 54.0 | 54.9 |

Table X. Analysis of Variance of Potato Yield Data from Table VIII: Normal and Late Planting; Single and Multiple Eyes 1951

| Variance due to - | DF | S.Sq. | M.Sq. | F | 5% | 1% |
|---|---|---|---|---|---|---|
| Replicates | 2 | 37.3 | 18.7 | .06 | 19.0 | |
| Dates | 1 | 352.2 | 352.2 | 10.4 | 18.51 | |
| Main plot error (1) | 2 | 67.5 | 33.8 | | | |
| Eyes | 1 | 10.2 | 10.2 | | | |
| Eyes x dates | 1 | 1.9 | 1.9 | | | |
| Error (2) sub-plot | 4 | 175.4 | 43.9 | | | |
| Total | 11 | 644.5 | | | | |

Table XI. Tuber Yields in 1950 from Shallow and Deep Planting; High, Low and No Hilling; Data in Pounds from 28 hills

| Planting depth | Hilling | Replicates I | II | III | Treatment means |
|---|---|---|---|---|---|
| | High | 14.5 | 24.0 | 20.0 | 19.5 |
| Shallow | Low | 18.0 | 23.0 | 24.0 | 21.7 |
| | None | 15.5 | 23.0 | 22.5 | 20.3 |
| | High | 20.0 | 20.5 | 22.0 | 20.8 |
| Deep | Low | 16.0 | 17.0 | 22.5 | 18.5 |
| | None | 23.5 | 17.0 | 14.0 | 18.2 |
| | Replicate means | 17.9 | 20.7 | 20.8 | 19.8 |

Table XII. Summary of Treatment Means from Table XI

| Planting depth | Degrees of hilling High | Low | None | Means |
|---|---|---|---|---|
| Shallow | 19.5 | 21.7 | 20.3 | 20.5 |
| Deep | 20.8 | 18.5 | 18.2 | 19.2 |
| Means | 20.1 | 20.1 | 19.3 | 19.8 |

Table XIII. Analysis of Variance of Potato Yield Data in Table XI: Shallow and Deep Planting; High, Low and No Hilling 1950

| Variance due to - | DF | S.Sq. | M.Sq. | F | 5% | 1% |
|---|---|---|---|---|---|---|
| Planting Depth | 1 | 8.00 | 8.00 | | | |
| Replicates | 2 | 33.08 | 16.54 | | | |
| Error (1) | 2 | 64.75 | 32.38 | | | |
| Hilling | 2 | 3.08 | 1.54 | | | |
| Hilling x depth | 2 | 16.75 | 8.38 | | | |
| Error (2) | 8 | 77.34 | 9.67 | | | |
| Total | 17 | 203 | | | | |

Table XIV. Tuber Yields in 1951 from Shallow and Deep Planting; High, Low and No Hilling; Data in Pounds from 27 hills

| Planting depth | Hilling | Replicates I | II | III | Treatment means |
|---|---|---|---|---|---|
| | High | 45 | 63 | 51 | 53.0 |
| Shallow | Low | 44 | 53 | 48 | 48.3 |
| | None | 46 | 52 | 55 | 51.0 |
| | High | 40 | 42 | 44 | 42.0 |
| Deep | Low | 37 | 39 | 43 | 39.7 |
| | None | 55 | 42 | 40 | 45.7 |
| Replicate means | | 44.5 | 48.5 | 46.8 | 46.6 |

Table XV. Summary of Treatment Means from Table XIV

| Planting depth | Degrees of hilling High | Low | None | Means |
|---|---|---|---|---|
| Shallow | 53.0 | 48.3 | 51.0 | 50.8 |
| Deep | 42.0 | 39.7 | 45.7 | 42.4 |
| Means | 47.5 | 44.0 | 48.3 | 46.6 |

Table XVI. Analysis of Variance of Potato Yield Data in Table XIV: Shallow and Deep Planting with High and Low Hilling 1951

| Variance due to - | DF | S.Sq. | M.Sq. | F | 5% | 1% |
|---|---|---|---|---|---|---|
| Planting depth | 1 | 312.5 | 312.5 | 4.2 | 18.51 | |
| Replicates | 2 | 48.5 | 24.3 | .3 | | |
| Error (1) | 2 | 148.0 | 74.0 | | | |
| Hilling | 2 | 63.5 | 31.8 | 1.2 | 4.46 | |
| Hilling x depth | 2 | 24.3 | 12.2 | .5 | 4.46 | |
| Error (2) | 8 | 213.5 | 26.7 | | | |
| Total | 17 | 810.3 | | | | |

# SOLANINE DETERMINATIONS

## METHODS OF ANALYSIS

A different method was used each of the two years to extract solanine from the frozen potato tissue samples. Once the solanine was obtained, however, the same colorimetric method was used each year for its estimation. To gain an understanding of methods of extraction, and to appreciate some of the difficulties inherent in such studies, a brief review is necessary.

### Fluorescence of Potato Tissue in Ultra-Violet Light

Dabbs (16) attempted to estimate solanine in pure solution or in tissue slices by fluorescence in ultra-violet light. He noted that all tuber slices showed slight fluorescence and that the region of maximum fluorescence was immediately under the tuber skin. However, he reports it was impossible to estimate differences in intensity of fluorescence.

Fluorescence of tuber slices and potato extracts evidently can be caused by several things. Allan (3) found fluorescence to occur in the presence of leaf roll virus, and it is also known to occur in the presence of *Corynebacterium*, *Fusarium* and *Pythium* species of organisms. Newton and Jones (40) report that frost or low temperature injury to potatoes can be detected by the fluorescence of the tuber slices under ultra-violet light, but they found acetone extracts of both normal and frozen potato tissue to be fluorescent. This latter effect

may have been due to the solubility of both solanine and solanidine in acetone. The former, however, precipitates out first, and, in fact, the preferential solubility of solanidine over solanine in acetone makes it possible to separate the two and isolate them in the pure state. Dabbs did not try acetone extracts, but it is unlikely that such extracts would show differences in fluorescence in relation to solanine content. The use of ultra-violet light for estimation of solanine can be discounted for two reasons. Not only does it fail to give differences in intensity of fluorescence (16), but also it is not specific for solanine only (3, 40).

## Chemical Analysis and Colorimetric Estimation

Many methods of solanine analysis in potato plants and tubers have been developed in the past. All of them are long and involve many steps for its extraction and estimation. The earlier workers such as Morgenstern (38), 1907, and Bomer and Mattis (7), 1924, used a gravimetric determination, extracting with dilute acetic acid solutions and expressing the extract with hydraulic pressure. Lepper (32), in 1938, used a gravimetric analysis involving the use of a dilute citric acid solution.

According to Pfankuch (42), 1938, the gravimetric method of Bomer and Mattis was the standard method up to that time. His chief criticism of the method is that it is a long

and tedious one designed to yield only pure solanine. It now appears, as Wolf and Duggar (55) have shown, that the most serious fault of this method was the arbitrary application of a solubility correction of 2.75 mg. solanine per 100 ml. of alkaline filtrate without checking the pH of the latter. Wolf and Duggar showed, in 1946, that no correction factor for solubility need be made if solanine were precipitated from an alkaline solution above a pH of 9.3. Frequently, with the method of Bomer and Mattis, the correction factor exceeded the amount of solanine actually recovered.

Connor (15), in 1938, used an acetic acid extraction method. The crude solanine precipitate is hydrolyzed with hydrochloric acid and the amount of solanine determined by a titrimetric determination of reducing sugars split off by hydrolysis. To this was added a solubility correction factor of 25 mg. per litre of filtrate. The method has two important limitations. It can only be used on relatively high concentrations of solanine solutions (at least 30 mg. per cent), and it determines only solanine but not solanidine and solanthrene. The food technologist, however, is interested in determining total alkaloids.

Also in 1938, Pfankuch, by adopting Alberti's sulphuric acid-formaldehyde color reaction to estimating solanine by colorimetry, overcame two difficulties at once: a solution of impure solanine can be used, from which the total alkaloids

solanine, solanidine and solanthrene can be determined quite accurately in amounts within concentrations of 10 to 50 mg. per cent, without reference to a curve. With concentrations above 50 mg. per cent, dilution is recommended for better accuracy. Pfankuch (42) states that either acetic acid or dilute sulphuric acid can be used successfully as solvents. A light-colored filter, wave length 530 mu is used because it does not interfere with possible yellow colors in the solution.

Pfankuch (42) states that the prescribed formaldehyde concentration has to be used very accurately as its influence on the intensity of the color is tremendous, stronger concentration giving a deeper color. Though he was satisfied with the accuracy of the colorimetric method of determining total alkaloids, Pfankuch was not satisfied with extraction methods available at that date. He also used a solubility correction factor of 2.5 mg. per 100 c.c. filtrate.

Rooke _et al._ (45), working in England in 1943, made a critical experimental study of available methods up to that date, and finally adopted a modified Pfankuch extraction technique using a solubility correction factor of .2 mg. per 100 ml. filtrate. Dabbs (16), in 1951, recognizing the importance of the discovery by Wolf and Duggar (55) that solanine is insoluble in aqueous solutions above pH 9.3, adopted Rooke's method of extraction but used the method of Wolf and Duggar for adjusting

the pH to 10.2 - 10.4 before precipitation. The resultant method he has termed the Standard Analytical Method. It involves no correction for solubility of solanine in the alkaline filtrate. Since this method was used for solanine extractions of the 1950 samples in the experiments described herein, it is outlined in detail below under the headings, "Cold Ethanol Extraction Method" and "Colorimetric Estimations."

Cold Ethanol Extraction Method

1. To thoroughly minced samples (100 - 150 gms.) of material add 150 ml. of 95 per cent ethanol, followed by 3 c.c. glacial acetic acid.
2. Stir well and keep 18 hours with occasional stirring.
3. Filter on a Buchner (Whatman's #4 paper).
4. A further two similar extractions of the residue are made with 150 ml. of 64 per cent ethanol (but no acetic acid).
5. Combined extracts are concentrated under reduced pressure to approximately 50 ml.
6. 5 gm. of $Na_2SO_4$ are added and the mixture warmed in a water bath for about one-half hour. A flocculent precipitate (mostly protein) forms.
7. The mixture is cooled, 2 ml. of 20 per cent $H_2SO_4$ added, and is filtered on a Buchner (Whatman's #4 paper). The residue is washed

with about 10 ml. of water.

8. The filtrate plus washings is adjusted to pH of 10.0 - 10.4 with concentrated $NH_4OH$.

9. The solanine is precipitated by digesting in a water bath for 20 - 30 minutes.

10. Filter through Whatman's #42 paper and wash the precipitate with about 10 ml. of 2 per cent ammonia.

11. The precipitate is dissolved in three or four 20 ml. increments of 0.5 per cent acetic acid, and made carefully to volume in a 100 ml. volumetric flask.

As stated above, this method was used in the analysis of the 1950 samples, but with slight modifications. After the filtrate plus washings is adjusted to the pH 10.0 - 10.4 with concentrated $NH_4OH$, it is transferred to a 250 ml. centrifuge flask, digested as above, and cooled. The precipitate is centrifuged for 20 minutes. Decant the filtrate as much as possible without disturbing the precipitate. The above modification was adopted to save time and difficulties in filtering where large amounts of filtrate are present.

Colorimetric Estimations

Colorimetric estimations of the total solanine*

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* Total solanine refers to solanine, solanidine and solanthrene collectively, and is hereafter designated as solanine.

present were then made according to Pfankuch's method (42) as modified by Wolf and Duggar (55). This method is briefly as follows:

1. Three millilitres of the .5 per cent acetic acid solution are measured out by precision pipette into a 50 ml. Erlenmeyer flask.
2. Six millilitres of concentrated sulphuric acid (C. P.) are added drop by drop while swirling the Erlenmeyer in an ice-bath.
3. The yellow solution which forms is allowed to stand for one minute.
4. Then three millilitres of 1 per cent formaldehyde are added drop by drop with cooling in ice-bath as before.
5. With the addition of the formaldehyde, the yellow color changes to orange, then red, and finally to violet-red.
6. Forty to fifty minutes after the last drop of formaldehyde is added, the maximum per cent absorption is read from the Fisher Electrophotometer, using a green filter with maximum light transmission at 525 mu.

Pfankuch (42) and Rooke et al (45) used a filter with maximum light transmission at 530 mu, while that employed by Wolf and Duggar (55) allowed maximum transmission at 540 mu.

All these workers allowed 90 minutes to elapse before taking readings. However, Dabbs (16) found maximum absorption to occur between 40 - 50 minutes after adding the last drop of formaldehyde, so readings were taken at that time.

Calculations

Two colors are run for each extraction and each extraction is done in duplicate so that at least four absorption readings are obtained for each sample. The mean of these four readings is converted to mg. solanine by multiplying by a Calibration Factor previously calculated with a pure solanine solution of known concentration. The Calibration Factor used was 0.4266, and the calculation is made on the basis of 100 gms. fresh weight of tuber.

## Soxhlet Extraction Methods

Before proceeding to the method used on the 1951 samples, it will be necessary to mention briefly the method published by Wolf and Duggar (55) in 1946. Essentially, it is as follows:

After three 1-hour extractions with .25 per cent acetic acid, the combined extracts are made slightly ammoniacal as soon as prepared to prevent enzyme activity, and a bit of diatomaceous earth added.

The extract is evaporated to dryness, then they proceed to re-extract the crude dried extract in a Soxhlet with 95 per cent ethanol for two 18-hour periods, regrinding the residue once between extractions. They state that Bomer and Mattis extracted in this manner for two 5-hour periods regrinding the residue once, while they report Valentin reground the residue three times for a total of 12 hours of Soxhlet extraction.

Dabbs (16) conceived the clever idea of using acidified ethanol for a single Soxhlet extraction period of 18 to 20 hours on a 25 gm. sample of the fresh tuber material. Only 5 drops of glacial acetic acid are added to 125 ml. of 95 per cent ethanol. This method was used for the 1951 samples.

In other ways, the method for obtaining the protein-free precipitate from the crude extract is essentially the same as described for the cold ethanol extraction method, except proportionally smaller amounts of reagents are used, fewer transfers are required, and the precipitate is made up to only 50 ml. Though Dabbs (16) claimed to be able to complete an extraction in duplicate and run the color determination in 24 hours, the procedure followed in the work outlined here allowed the solanine to precipitate overnight in the refrigerator, and also allowed the washed precipitate and filter-paper to become absolutely dry before making up to volume for colorimetric estimation. Also solutions of solanine and of 1 per cent

formaldehyde reagent were not allowed to stand longer than overnight prior to making determinations, and preferably were made up the same day.

## SOLANINE RESULTS - 1950

The long method gave unsatisfactory results. It required about 5½ days to complete duplicate analyses on one sample, and when more samples were run simultaneously a longer time elapsed. Repeated analyses often failed to give satisfactory checks.

Results obtained are tabulated in Tables XVII and XVIII. No treatment trends are evident from the data. No analysis of this data has been attempted as it was felt that too much uncontrolled error existed in many cases between individual estimations on a single sample. It is believed that due to the method employed, the extraction of many samples was either incomplete or there was a loss of solanine by decomposition when the extract was allowed to remain in an acid condition for long. Wolf and Duggar (55) made the dilute acetic acid extracts ammoniacal as soon as prepared. A similar procedure with the acidified cold ethanol extracts might have given more uniform results for the 1950 samples.

## SOLANINE RESULTS - 1951

### Estimated Solanine Concentrations Resulting from Normal and Late Planting with Single and Multiple Eyes

The data for 1951 are presented in Table XIX according to treatments and replicates, while Table XX summarizes these results according to treatments only.

### Discussion

Two features of this data are worth noting:

(1) The rather consistent trend toward different solanine concentrations for each replicate, regardless of cultural treatments.

(2) The tendency for late-planted multiple eyes to yield tubers with less solanine and for normal-planted multiple eyes to yield tubers with more solanine. In other words, the additional shading that may have been provided by multiple-eye sets over that provided by single-eye sets, while it appears to have resulted in slightly lower mean solanine concentrations in late plantings, showed a reverse tendency in normal plantings. The above-mentioned features are accented in the split-plot analysis of variance table (Table XXI), where the tendency for the single eyes to behave in a manner opposite to the multiple-eyes, results in a mean square of zero for eyes alone but a rather

large variance due to interaction between eyes and dates of planting. The latter variance is not significant, however, nor is the variance due to replicates.

## Estimated Solanine Concentrations Resulting from Shallow and Deep Planting with High, Low and No Hilling

These data are presented according to treatments and replicates in Table XXII and summarized according to treatments only in Table XXIII.

## Discussion

The tendency for solanine concentrations to vary more with the replicate than with the treatment is even more marked than in the previous experiment. In fact, the split-plot factorial analysis of variance table (Table XXIV) shows the variance due to replicates significant at the 5 per cent level. Two other less notable trends are the higher solanine concentrations found in the shallow planting, especially with no hilling, though deep planting showed a similar trend.

The results of this experiment are just the opposite to what was expected. It was thought that replicate variation would be small, but that treatments which caused or allowed tubers to form and remain close to the surface of the soil would result in significantly higher solanine concentrations due to less soil

coverage on the tubers and consequent greater solanine synthesis from solar radiation.

Solar radiations, especially if high in ultra-violet light could conceivably cause higher solanine concentrations in the growing potato tubers, as Conner (15) has shown that solanine synthesis does occur in tubers exposed to ultra-violet light, without the concurrent greening. One would expect to find solar radiations high in ultra-violet energy in regions of clear skies, especially at higher altitudes where less filtering from the atmosphere occurs.

Depth of soil cover, type of soil and amount of shading by the potato tops also could be factors which might influence the amount and quality of solar radiations which the growing tubers may receive.

Table XXIII shows that a slight increase in the mean solanine concentration occurred in the shallow-planted sets that received no-hilling treatment. Deep-planted sets similarly treated were scarcely affected. In the remaining plots of the experiment where hilling was practiced, the mean solanine concentrations for shallow planting was no greater than for deep planting. Nor was there any difference between low hilling and high hilling.

These effects result in variation due to hilling being noticeable but not significant (Table XXIV).

## Conclusions

On the basis of the significance of replicate variance in the latter experiment, it is concluded that replicates receiving the same cultural treatments show significant differences in solanine content. Therefore, the cultural treatments studied did not have as great an effect on the occurrence of solanine in the tubers as did other conditions of the environment. The high replicate variance indicates that the soil environment may be a much more decisive factor in affecting solanine concentrations than was hitherto supposed. The outcome of the experiment stresses the importance of having uniform field conditions on which to apply the experimental design.

The factorial experiments have shown that the cultural practices studied are not major factors in the occurrence of solanine in growing potatoes. Except for the early and late planting, these factors were designed primarily to check on the possible effects of solar radiation upon solanine synthesis in the potato tubers.

It was found, in the 1951 season, under Edmonton conditions, that with normal soil cover and top growth the effect of solar radiation on solanine synthesis in the tubers is negligible. It was also found that the more immature tubers from the late planting do not contain more solanine than the more mature tubers from normal planting.

The discussion could be terminated conveniently at this point. However, a further dissertation is presented (see page 75) with the hope that it may provide valuable clues for the ultimate solution of the problem of solanine bitterness in table potatoes.

Table XVII. Estimates of Solanine* in Tubers in 1950 from Shallow and Deep Planting with High, Low and No Hilling, Using the Cold Ethanol Extraction Method

| Treatments | | REPLICATES | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | I | | | II | | | III | | |
| Planting | Hilling | Range | N | Mean | Range | N | Mean | Range | N | Mean |
| | High | 1.85-2.50 | 2 | 2.18 | 3.04-3.10 | 2 | 3.07 | 7.76-8.96 | 2 | 8.36 |
| Shallow | Low | 3.83-6.91 | 2 | 5.37 | 2.47-6.31 | 2 | 4.39 | 2.28-2.39 | 2 | 2.34 |
| | None | 2.71-7.65 | 2 | 5.18 | 3.40-5.94 | 2 | 4.67 | 2.70-9.10 | 5 | 6.61 |
| | High | 3.24-11.98 | 4 | 3.73 | 2.88-4.80 | 2 | 3.84 | 0.80-4.69 | 4 | 2.66 |
| Deep | Low | 1.53-10.44 | 3 | 5.71 | 2.19-6.06 | 2 | 4.13 | 5.49-5.77 | 2 | 5.63 |
| | None | 1.12-7.74 | 3 | 3.35 | 4.66-4.69 | 2 | 4.68 | 8.11-8.50 | 2 | 8.31 |

* All estimates expressed in milligrams solanine per 100 gm. fresh tuber.

Table XVIII. Estimates of Solanine* in Tubers in 1950 from Normal and Late Planting of Single and Multiple Eyes, Using the Cold Ethanol Extraction Method

| Treatments | | REPLICATES | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | I | | | II | | | III | | |
| Planting date | Eyes in seed-piece | Range | N | Mean | Range | N | Mean | Range | N | Mean |
| Normal | Single | 7.21-7.24 | 2 | 7.23 | 4.46-7.57 | 4 | 6.84 | 6.03-11.02 | 3 | 9.29 |
| | Multiple | 7.06-7.27 | 2 | 7.17 | 8.53-9.81 | 2 | 9.17 | 10.95-11.21 | 2 | 11.06 |
| Late | Single | 4.41-9.17 | 6 | 6.63 | 2.74-8.62 | 3 | 5.99 | 2.56-7.99 | 4 | 4.28 |
| | Multiple | 2.06-4.65 | 6 | 3.51 | 9.57-10.30 | 3 | 9.87 | 2.96-5.61 | 4 | 4.55 |

* All estimates expressed in milligrams solanine per 100 gm. fresh tuber.

Table XIX. Estimates of Solanine* in Tubers in 1951 from Normal and Late Planting of Single and Multiple Eyes, Using the Soxhlet Extraction Method

| Planting date | Eyes in seed-piece | Replicates I | II | III | Treatment means |
|---|---|---|---|---|---|
| Late | Single | 9.00 | 7.21 | 10.40 | 8.87 |
| | Multiple | 7.93 | 6.34 | 7.35 | 7.20 |
| Normal | Single | 5.91 | 7.28 | 10.37 | 7.85 |
| | Multiple | 9.73 | 7.62 | 11.73 | 9.79 |
| Replicate means | | 8.25 | 7.11 | 9.96 | 8.41 |

* All estimates expressed in milligrams solanine per 100 gm. fresh tuber.

Table XX. Summary of Treatment Means from Table XIX

| Planting date | Eyes in seed-piece Single | Multiple | Means |
|---|---|---|---|
| Late | 8.87 | 7.20 | 8.04 |
| Normal | 7.85 | 9.79 | 8.77 |
| Means | 8.36 | 8.45 | 8.41 |

Table XXI. Analysis of Variance of Solanine Data in Table XIX: Normal and Late Planting of Single and Multiple Eyes, 1951

| Variance due to - | DF | S.Sq. | M.Sq. | F | 5% | 1% |
|---|---|---|---|---|---|---|
| Replicates | 2 | 16.66 | 8.33 | 4.19 | 19.0 | |
| Dates | 1 | 1.62 | 1.62 | | | |
| Error (1) | 2 | 3.98 | 1.99 | | | |
| Eyes | 1 | 0.0 | 0.0 | | | |
| Eyes x dates | 1 | 9.14 | 9.14 | 5.74 | 7.71 | |
| Error (2) | 4 | 6.36 | 1.59 | | | |
| Total | 11 | 36.14 | | | | |

Table XXII. Estimates of Solanine* in Tubers in 1951 from Shallow and Deep Planting with High, Low and No Hilling, Using the Soxhlet Extraction Method

| Planting depth | Hilling | Replicates I | II | III | Treatment means |
|---|---|---|---|---|---|
| | High | 3.29 | 7.70 | 13.04 | 8.01 |
| Shallow | Low | 3.75 | 5.13 | 14.04 | 7.64 |
| | None | 5.83 | 9.28 | 16.28 | 10.46 |
| | High | 6.60 | 7.38 | 9.44 | 7.81 |
| Deep | Low | 5.72 | 5.56 | 12.23 | 7.84 |
| | None | 3.92 | 4.97 | 15.89 | 8.26 |
| Replicate means | | 4.85 | 6.67 | 13.49 | 8.34 |

* All estimates expressed in milligrams solanine per 100 gm. fresh tuber.

Table XXIII. Summary of Treatment Means from Table XXII

| Planting depth | Degrees of hilling High | Low | None | Means |
|---|---|---|---|---|
| Shallow | 8.01 | 7.64 | 10.46 | 8.70 |
| Deep | 7.81 | 7.84 | 8.26 | 7.97 |
| Means | 7.91 | 7.74 | 9.36 | 8.34 |

Table XXIV. Analysis of Variance of Solanine Data in Table XXI: Normal and Late Planting of Single and Multiple Eyes, 1951

| Variance due to - | DF | S.Sq. | M.Sq. | F | 5% | 1% |
|---|---|---|---|---|---|---|
| Planting depth | 1 | 2.45 | 2.45 | | | |
| Replicates | 2 | 248.68 | 124.34 | 31.16* | 19.0 | 99.0 |
| Error (1) | 2 | 7.98 | 3.99 | | | |
| Hilling | 2 | 9.56 | 4.78 | 1.22 | 4.46 | |
| Hilling x depth | 2 | 4.95 | 2.48 | | | |
| Error (2) | 8 | 31.25 | 3.91 | | | |
| Total | 17 | 304.87 | | | | |

* Replicates significant at the 5% level.

## OTHER FACTORS OF THE ENVIRONMENT

Solanine is undoubtedly present to a more or less degree in potatoes everywhere. It is also quite evident that certain conditions of growth and storage result in the occurrence of higher concentrations. Obviously, there must be some specific factor or combination of factors of the environmental field conditions that contribute to higher solanine concentrations in the tubers. Rather than a combination of factors, it may sometimes be the result of a succession of factors or conditions. The reason why some potatoes "become" bitter in storage is very likely due to a higher-than-normal initial solanine content, plus a normal synthesis or accumulation in storage. Occasionally, samples are extremely high in solanine when dug. The literature relating to the effect of storage has been reviewed. It is beyond the scope of this study to delve into the causal factors of apparent solanine synthesis in storage.

Why, then, do some potato samples contain too much solanine when dug? The problem must be tied up with the nitrogen and carbohydrate metabolism of the potato plant.

### Biochemical and Physiological Considerations

Street and co-workers (49) made a study of the nature and distribution of nitrogen in the potato plant. They state that solanine is part of the possible mobile nitrogen.

This mobile nitrogen, that form most actively involved in translocation, they classified as "other nitrogen." "Other nitrogen" equals total non-protein nitrogen minus the sum of ammonia, amide, amino and nitrate nitrogen. The basis for their belief that "other nitrogen" is the mobile form in the potato was that they found what they called a positive concentration gradient increasing as they progressed from roots to stems to petioles to leaves.

"Other nitrogen" is quantitatively an important fraction of the non-protein nitrogen in all parts of the plant. It is complex in composition and will include:

polypeptide and peptide nitrogen;

purine nitrogen, i.e. xanthine, hypoxanthine, quanine, adenine;

nitrogen of the simple bases - choline, cadaverine, narcotine, acetylcholine;

non-amino nitrogen of arginine, histidine, proline and tryptophane; and

total solanine nitrogen, which is quantitatively an unimportant fraction of this mobile nitrogen.

Choline makes up an important part of the simple natural bases in the potato, and it is postulated that it functions in biological methylations generally, and alkaloidal synthesis particularly.

Street and co-workers (49), to convert solanine to solanine nitrogen, multiplied by the factor .016. In fact, they say that the values recorded for solanine content, if expressed in terms of nitrogen, represent only a very minute fraction of the "other nitrogen."

Conversely, then, only 1 mg. of choline nitrogen or any other fraction of mobile nitrogen, if entirely converted to solanine nitrogen in solanine will become 62.5 mg. solanine, which is two to three times the upper limit of safety and which is about ten times that normally found in potatoes. So it can readily be seen that the slightest physiological unbalance could readily produce very high solanine with resultant bitterness in potatoes.

This physiological upset might conceivably be caused by some environmental factor or factors which may be difficult or impossible to control under field conditions. In the Netted Gem variety there may be a genetical predisposition towards this upset, say under certain conditions of the environment, expecially at a certain developmental stage.

The manifestation of the physiological upset is large amounts of solanine in the potatoes when dug. Whether the disorder involves abnormal synthesis of solanine in the tuber or translocation of excessive amounts to the tuber is of little concern at the moment. The net result is the same. Such physiolog-

ical disturbances may be caused by a number of factors:

(a) Mechanical injuries caused by frost and hail.

There is evidence that the occurrence of damage to the plants from summer hail and frost increases the incidence of solanine in this variety especially. Frequently, when the history of a bitter sample is investigated, it is found that one or both of these phenomena are involved. That frozen tops can cause higher solanine in the tubers is more than mere speculation, as seen by Table XXV, which shows the results of successive solanine determinations on samples of Netted Gem potatoes taken at weekly intervals from September 3 to October 1.

Table XXV. Results of Solanine Determinations Made on Single Hills Dug at Weekly Intervals from Guard Rows Receiving Treatment Indicated

| | Shallow Planting, No Hilling | | | Deep Planting, Low Hilling | | |
|---|---|---|---|---|---|---|
| Date | No. Tubers | Wt. of tubers in hill in gms. | Solanine content in mgs.per 100 gms. | No. tubers | Wt. of tubers in hill in gms. | Solanine content in mgs. per 100 gms. |
| Sep.3 | -- | -- | -- | 7 | 1177 | 0 |
| do 10 | -- | -- | -- | 7 | 1046 | Trace |
| do 17 | 4 | 1666 | 1.2 | 9 | 1560 | 0 |
| do 24 | 5 | 1698 | 3.58 | 10 | 2060 | 6.65 |
| Oct. 1 | 12 | 1681 | 1.96 | 7 | 946 | Trace |
| do 1 | 3 | 614 | 8.28 | -- | -- | -- |

The most noteworthy thing about the data is that little or no solanine was found in the potato samples taken before the killing frost on September 22. Samples taken on September 24, two days after the killing frost, had a heavy protein precipitate and solanine was found in both samples in some quantity for the first time. However, two out of three of the samples taken a week later showed decreased solanine content again. The higher solanine in the third sample may be related, in part, to the small number of tubers in the hill and small total yield of the hill. It also may be related to proximity of tubers to the surface. It was noted that one tuber was sunburned at the tip. What caused the sudden rise in solanine in the tubers after the killing frost is difficult to say, unless the solanine moved down from the frozen tops by simple diffusion. Two other possibilities are that it was translocated from the unfrozen portion of the stem that was protected by soil, or that it moved out of the roots into the tubers.

That a killing frost appears to cause sudden increases in solanine concentration of tubers attached to frozen tops has been demonstrated. Whether this sudden rise is only temporary is uncertain. (Table XXV.) Light summer frosts and hail damage to the tops could conceivably cause even a greater physiological upset because the plant remains alive. A succession of such phenomena is not unknown and might result in a highly abnormal cumulative concentration of solanine in the tubers.

(b) Mineral deficiencies or nutritional unbalance.

A physiological disturbance caused by a lack of a major or minor element or an excess of one in relation to availability of others is not impossible. In fact, the high replicate variance, especially in the hilling experiments, points rather to a difference in available nitrogen or other major element (e.g., P or K). Since nitrogen is directly involved in the formation of the solanine molecule, it seems the most likely of the three. As with the *Nicotiana* spp. and *Cinchona ledgeriana*, high nitrogen levels may result in higher alkaloid production in the potato.

It must not be assumed at once, however, that excess nitrogen is the cause of abnormal solanine in the tuber. A sample of very bitter tubers was received from Leedale, Alberta, in October, 1951, which were reported to have caused sore throats when eaten. Analysis showed an estimated solanine content ranging from 50 - 68 mg. per 100 gm. This concentration is dangerously high. A quick soil test of available nutrients revealed that the soil in which the potatoes were grown was abnormally high in phosphorus and relatively high in potash. The figures in p.p.m. for the three major elements N, P and K were 1, 10 and 35, respectively. Compare this with other random analyses of garden soils of 6 - 0.5 - 30, or 5 - 0.5 - 5, analyzed at the same time. The disproportional amounts of available major elements merit closer study as possible factors contributing to solanine bitterness in Netted Gem potatoes.

The history of the potato crop in question was normal otherwise, except that hail damage had been suffered during the season.

These two bits of evidence lend strong support to the theory of a physiological disturbance caused by mechanical injuries or nutritional unbalance or a combination of the two.

## GENERAL SUMMARY AND CONCLUSIONS

Netted Gem potatoes were grown for two seasons in two split-plot factorial field cultural experiments. The factors studied were normal and late planting with single and multiple eyes, and deep and shallow planting with three levels of hilling.

Yield data and tuber samples for solanine analysis were taken each year at digging time. To offset any complication of solanine results by different storage histories, the 1951 tuber samples were minced in a Waring Blendor and quick-frozen one month after digging.

A modification of the cold alcohol extraction method of Rooke was used on the 1950 samples, but was found unsatisfactory. The new acidified ethanol Soxhlet extraction method of Dabbs proved very satisfactory with the 1951 samples. No solubility correction factor was used in either method; the pH of the solanine filtrate being adjusted to 10.2 to 10.4 after the method of Wolf and Duggar.

It is possible that the cold ethanol extraction method (modified Rooke method) might have given more reliable results if the combined extracts had been made alkaline as soon as prepared, as Wolf and Duggar did with the dilute acetic acid extraction.

Solanine concentrations were estimated colorimetrically with a Fisher Electrophotometer from a 0.5 per cent acetic acid

solution of the final precipitate according to the method of Pfankuch as modified by Wolf and Duggar.

No treatment or other trends were evident in the solanine results for 1950. The 1951 samples showed an average solanine concentration in mg. per 100 gms. fresh weight of 8.34 - 8.40 for the two experiments. These results compare very well with figures reported by other reliable sources, but were higher than those reported for the variety under Wisconsin conditions.

A statistical analysis was made of the solanine estimations for the 1951 treatments. A marked, though not significant, interaction existed between the time of planting and number of eyes in the seed-pieces. Lower mean solanine concentrations were found in the tuber samples from multiple eyes planted late and single eyes planted at normal time.

A high replicate variance for solanine concentration was found in both experiments in the 1951 season. In the depth of planting experiment with three levels of hilling, there were significant differences in solanine concentration between replicates.

Normal planting gave consistently, but not significantly, greater yields than late planting both years. No consistent differences were found in the yields obtained from single and multiple eyes. Shallow planting gave yields equal to or

greater than deep planting, though differences were not significant. Depth of hilling had no effect on yield either of the two years.

On the basis of the experiments reported herein, it is concluded that the cultural treatments studied had no significant effect on the occurrence of solanine in Netted Gem potatoes, nor upon the tuber yields.

On the basis of the significant replicate variance for solanine concentrations, it is further concluded that the uncontrolled factors of the environment in one experiment had a greater influence upon the solanine concentrations than did the cultural practices studied.

It is established that solanine, if present in the tuber in sufficient amounts, results in a bitter flavor. It is suggested that solanine bitterness may be a non-parasitic physiological disorder, to which the Netted Gem variety is extremely susceptible. It is postulated, with some supporting evidence, that such a physiological disorder might result from a nutritional unbalance, or from mechanical injuries as from frost or hail or both.

It is shown that if as minute a quantity as only one mg. of nitrogen is entirely converted to solanine nitrogen, it will result in 62.5 mg. of solanine, so that a normal balance in the carbohydrate-nitrogen metabolism of the potato plant is extremely important.

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# APPENDIX I

## Potato Taste Panel

A taste test was conducted on potato samples from cultural treatment lots selected for their representative solanine levels based on chemical analysis. The purpose was to determine if there was any correlation between the estimated solanine and degree of bitterness. Two tubers were pressure-cooked from each treatment, skins intact, then sampled for bitterness. In spite of the fact that the chemically analyzed samples were quick-frozen in November, while the taste-tested tubers were cooked on March 17, there was a marked correlation between estimated solanine and apparent bitterness. The results of the taste panel are shown in Table XXVI.

Table XXVI. Results of Potato Taste Panel

| Sample no. | Cultural treatment | Replicate no. | Estimated solanine concentration | Degree of bitterness | Other comments |
|---|---|---|---|---|---|
| 1 | Deep planting, no hilling | III | 15.89 | Fairly bitter | Rated bitterest by some. Second only to #2. Bitterness mostly in cortical area |
| 2 | Shallow planting, no hilling | III | 16.28 | Fairly bitter | Most bitter of the 6 samples according to about half the panel. |
| 3 | Shallow planting, no hilling | II | 9.28 | No bitterness to slightly bitter | |
| 4 | Shallow planting, low hilling | II | 5.13 | No observable bitterness | |
| | Deep planting, low hilling | II | 5.56 | | |
| 5 | Late planting of single eyes | III | 10.40 | Slightly bitter | Varied from no bitterness to slightly bitter. |
| 6 | Normal planting of single eyes | III | 10.37 | Slightly bitter (by most) | Placed in same class as 1 and 2 by several. Last tasted and therefore difficult to judge. |

# APPENDIX II

## Solanine Analyses on Tubers from a Potato Fertilizer Experiment

### Materials and Methods

In 1950, potato samples were taken at digging time from the treatment rows of a fertilizer trial at the Provincial Horticultural Station, Brooks, Alberta. The land is a sandy loam which had been in alfalfa hay production for the two previous seasons. All crops on this land are irrigated, the potatoes having received two irrigations in 1950. The pipe and furrow method of irrigation was used. Planting was done on June 5, and samples were dug October 4.

Four 6-ounce tubers were selected from each treatment, minced in the Waring Blendor and frozen in quadruplicate 150-gram samples in November, 1950. The frozen samples were not analyzed until February, 1952, when they were thawed and 25-gram samples extracted in duplicate by Dabbs' acidified ethanol Soxhlet extraction method. The solanine was estimated colorimetrically as previously described. The results are tabulated in Table XXVII.

## Results

Table XXVII. Estimates of Solanine* Concentrations in Tubers from a Fertilizer Experiment

| Treatment | Replicates I | II | III | Treatment means |
|---|---|---|---|---|
| Check | 10.9 | 11.2 | 5.2 | 9.1 |
| 11-48 at 200 lb./acre | 8.7 | 8.8 | 9.1 | 8.9 |
| 11-48 at 200 lb./acre + M | 9.1 | 5.4 | 11.4 | 8.6 |
| Replicate means | 9.6 | 8.5 | 8.6 | 8.9 |

* All estimates of solanine are expressed in milligrams solanine per 100 gm. fresh tuber.

## Discussion and Conclusion

An analysis of variance of the data treated as a randomized block design (which it was) showed no significant differences in solanine concentrations for treatments or replicates. Results are in agreement with those of most other workers (7, 9, 32) inasmuch as the fertilizers used had no effect on the solanine content of the tubers. These conclusions should not be accepted as final. The results of this and other experiments previously discussed indicate the desirability of conducting further studies on the effect of nutrition on solanine in potatoes, especially where the relative availability of the major elements required for growth is known and correlated with solanine content of the tubers.